

12-11-96

DATA EVALUATION REPORT

KRESOXIM-METHYL

STUDY TYPE: CHRONIC ORAL TOXICITY FEEDING - RAT (83-1a)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
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Prepared by

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DATA EVALUATION RECORD

STUDY TYPE: Chronic Oral Toxicity Feeding - Rat
OPPTS 870.4100 [83-1a]DP BARCODE: D225934SUBMISSION CODE: S504279P.C. CODE: 129111TOX. CHEM. NO.: noneTEST MATERIAL (PURITY): Reg. No. 242 009 (KRESOXIM-METHYL)
(92.7-95.6% w/w)SYNONYMS: BAS 490F; alpha-(methoxyimino)-2-((2-methylphenoxy)methyl) benzeneacetic acid, methyl esterCITATION: Mellert, W. (1994) Toxicology report. Chronic toxicity Study with Reg. No. 242 009 (BAS 490F) in rats: Administration in the diet for 24 months. BASF Aktiengesellschaft, Department of Toxicology, D-67056 Ludwigshafen/Rhine, FRG. BASF Registration Document Number 94/10951, Project Number 70C0180/91007, October 24, 1994. MRID 43864247. Unpublished.

Polloth, C. (1994) Toxicology Report. S-Phase Response with Reg. No. 242 009 in Rats After Administration in the Diet for 3 Weeks. BASF Aktiengesellschaft Department of Toxicology, D-67056 Ludwigshafen/Rhine, FRG. Report number 94/10922, October 5, 1994. MRID 43864246. Unpublished.

SPONSOR: BASF Corporation, Agricultural Products Group, Research Triangle Park, NC 27709-3528.EXECUTIVE SUMMARY: In a chronic toxicity study (MRID 43864247) Reg. No. 242 009 (92.7-96.6% w/w, Lots No. N 27 (IIIa1), N 30 (IIIa2), N 36 (IIIc1)) was administered to 20 Wistar rats/sex/dose in the diet at dose levels of 0, 200, 800, 8000, or 16,000 ppm (0, 9, 36, 370, and 746 mg/kg/day for males and 0, 12, 48, 503, 985 mg/kg/day for females, respectively) for 24 months.

There was no effect on mortality in either sex of rats. The body weights of 8000 and 16,000 ppm females were decreased throughout the study (about 3-10%; $p \leq 0.05$ or 0.01). Body weight gains paralleled body weight changes in both sexes; the changes were of minor biological significance. The level of serum gamma-glutamyl transferase (SGGT) was elevated 4.8 to 46-fold in 8000 and 16,000 ppm males throughout the study ($p \leq 0.01$; time and dose-dependent). Organ weight changes in 8000 and 16,000 ppm males included a dose-related increase in absolute and/or relative liver weight (16-22%, $p \leq 0.05$ or 0.01). The liver weight changes were correlated with the increased SGGT level in males and with numerous macroscopic and microscopic pathologies in both sexes, implicating the liver as a target organ. There was a dose-related increase in the incidence of liver cysts in 16,000 ppm males ($p \leq 0.01$) and minor increases in liver cysts and masses in both sexes at 8000 and 16,000 ppm ($p > 0.05$). These were correlated in males with an increased incidence of liver eosinophilic foci, mixed cell foci, and cellular hypertrophy at 8000 and/or 16,000 ppm

and in females with cellular hypertrophy at 16,000 ppm ($p \leq 0.05$ or 0.01).

The LOEL for both male and female rats is 8000 ppm (about 370 mg/kg/day in males and 503 mg/kg/day in females) under the conditions of this study. This is based in males on the increase in SGGT levels, liver weight and histopathological changes, and in females on the roughly 10% lowered body weights and weight gains throughout much of the study. The NOEL for both sexes is 800 ppm, corresponding to about 36 mg/kg/day in males and 48 mg/kg/day in females.

There was a dose-related increase in the incidence of hepatocellular carcinoma in both males and females at 8000 and 16,000 ppm (males: 0/20, 1/20, 1/20, 3/20, 8/20; females: 1/20, 0/20, 2/20, 6/20, 6/20 at 0, 200, 800, 8000, and 16,000 ppm, respectively. Although the increase was statistically significant only in 16,000 ppm males ($p \leq 0.01$), it was most likely treatment-induced in 8000 ppm females as well. This is supported by positive results in a chronic oncogenicity study at the same doses and strain of rats. The relationship to treatment of the sporadic liver cholangioma and/or cholangiocarcinoma (0 to 2 animals/group) in unknown.

This chronic toxicity study is classified as acceptable, and satisfies the guideline requirement for a chronic oral study (83-1a) and carcinogenicity study (83-2a) in rats.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided. With respect to the GLP statement, the study meets the requirements for 40 CFR 160 but differs in that it was conducted in accordance with the GLP-Provisions of the Chemikaliengesetz (Chemical ACT; Bundesgesetzblatt 1990, Teil I, 22.03.90; FR Germany) and with the OECD Principles of Good Laboratory Practice (Paris, 1981).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Reg. No. 242 009

Description: solid whitish-brown powder

Lot/Batch #: Test substance numbers 91/180, 91/180-1, 91/180-2.
Batch numbers were N 27 (IIIa1); N 30 (IIIa2); and N 36 (IIIc1), respectively.

Purity: 94% (N27); 96.6% (N30); 93.7% (N36) (w/w; measured before use)

Stability of compound: stable at room temperature for at least 32 days

CAS #: 143390-89-0

2. Vehicle and/or positive control

none

3. Test animals

Species: rat

Strain: Wistar (Chbb: THOM (SPF))

Age and weight at study initiation: 42 days old; males: 181-213 g; females: 134-165 g

Source: Dr. Karl Thomae GmbH, Biberach/Riss, FRG

Housing: Animals were housed singly in type DK III stainless steel wire cages with a floor area of about 800 cm²

Diet: Animals were fed the ground KLIBA maintenance diet rat/mouse/hamster, 343 meal (supplied by Klingentalmuehle AG, Kaiseraugst, Switzerland) *ad libitum*.

Water: Drinking water was available *ad libitum*

Environmental conditions:

Temperature: 20-24 °C

Humidity: 30-70%
Air changes: not specified (fully air-conditioned)
Photoperiod: 12 hours light, 12 hours dark per 24 hour period
Acclimation period: 10 days for males; 9 days for females

B. STUDY DESIGN

1. In life dates

Start: May 23, 1991 (males); June 5, 1991 (females)
end: July 1, 1993

2. Animal assignment

Animals were assigned randomly, based on their weights, to the test groups in Table 1.

TABLE 1: Study design					
Test Group	Conc. in Diet (ppm)	Mean dose to animal (mg/kg/day) ¹		Number of animals	
		Male	Female	Male	Female
0 (Control)	0 (Control)	0	0	20	20
1	200	9	12	20	20
2	800	36	48	20	20
3	8000	370	503	20	20
4	16,000	746	985	20	20

Data taken from pp. 24 and 45, MRID 43864247.

¹Values calculated as time-weighted averages from the consumption and body weight gain data over the entire 24-month administration period.

3. Dose selection rationale

Dose selection was based on the results of previous 4-week (Project No. 30S0055/90002) and 3-month (MRID 43864245) studies using the same strain of rats. In the 4-week study, increased gamma-glutamyltransferase and albumin levels were found in males at the highest dose tested (16,000 ppm or about 1455 mg/kg body weight). In the 3-month study, one or more parameters were altered at 2000, 8000, and 16,000 ppm in one or both sexes (body weight, serum enzyme levels, and relative liver weight). The 16,000 ppm dose resulted in an intake of 1170 mg/kg body weight for males and 1374 mg/kg/day for females, which is roughly the highest dose recommended (1000 mg/kg/day) for testing according to EPA guidelines.

4. Diet preparation and analysis

Diet was prepared at intervals for which the stability of the test substance in the diet was guaranteed (32 days) by mixing appropriate amounts of test substance with ground KLIBA maintenance diet rat/mouse/hamster,

343 meal. It was stored at room temperature. The stability of the test substance at room temperature was established in a previous experiment using a 50 mg/kg sample. Homogeneity was tested at the beginning of the administration period at 200 and 16,000 ppm (6 samplings at each dose). During the study, samples of treated food were analyzed approximately every three months for their concentration.

Results -

Homogeneity Analysis: 100-107% at 200 ppm; 93.0-100% at 16,000 ppm

Stability Analysis: At day 10, 101-102% of initial value; at day 32 97.0-99.1% of initial value

Concentration Analysis: Relative to the target concentration, values varied from 94.9-108.0% at 200 ppm; 91.0-107.8% at 800 ppm; 93.2-101.7% at 8000 ppm, and 93.0-101.4% at 16,000 ppm over the 2 years of testing (9 sampling dates).

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

5. Statistics

A parametric one-way analysis of variance via the F-test (ANOVA) and Dunnett's test (two-sided) were used to make simultaneous comparisons of the test groups with the control group for the body weight, body weight change, serum enzymes, blood chemistry, and all hematology parameters except the differential blood count. For all urinalysis parameters except turbidity and color, the pairwise comparison of treated and control groups was done using Fisher's exact test (one or two-sided). The terminal body weight in the pathology report, as well as the absolute and relative (to body) organ weights, were statistically evaluated using Dunnett's test for simultaneous comparison of dose and control groups. The Chi-square test was used to analyze pairwise differences between control and treated groups' histopathological findings, although only the results of the statistical analysis for liver lesions were reported. The pathology statistical calculations were performed using the Roelee® statistical program. No statistical analyses were reported for the food consumption, food efficiency, test substance intake, differential blood count, urine, or the incidence of gross and most microscopic lesions (pathological results were statistically analyzed by the reviewer using the Fisher exact test).

C. METHODS

1. Observations

Animals were inspected twice a day on Monday-Friday, and once a day on Saturdays, Sundays, and holidays for signs of toxicity and mortality. Additional comprehensive clinical examinations and palpations of the animals were carried out approximately weekly.

2. Body weight

Animals were weighed before the start of the administration period, once a week during the first 13 weeks of the administration period, and every four weeks thereafter until the end of the study.

3. Food consumption and compound intake

Food consumption for each animal was determined once per week for the first 13 weeks of the study and over a period of one week at 4-week intervals until the end of the study. The food consumption was calculated as g food/animal/day. Food efficiency (body weight gain in g/food consumption in g per 7 days X 100 for weeks 1-13; g per 28 days/[food consumption in g per 7 days x 4] X 100 for weeks 14 until end of study) and compound intake (mg/kg/day) values were calculated as time-weighted averages from the consumption and body weight gain data of individual animals.

4. Ophthalmoscopic examination

Eyes were examined with an ophthalmoscope before the start of the study and at the end of the administration period in the control and highest dose groups (16,000 ppm)

5. Blood was collected at approximately months 3, 6, 12, 18, and 24 from the retro orbital venous plexus of all surviving animals for hematology and clinical analysis (this corresponded to days 92, 179, 365, 547, and 732 in males and 92, 180, 365, 548, and 729 in females). Blood was collected in the morning with no prior fasting or anesthesia. The CHECKED (X) parameters were examined.

a. Hematology

x	Hematocrit (HCT)	x	Leukocyte differential count
x	Hemoglobin (HGB)		Mean corpuscular HGB (MCH)
x	Leukocyte count (WBC)	x	Mean corpusc. HGB conc. (MCHC)
x	Erythrocyte count (RBC)		Mean corpusc. volume (MCV)
x	Platelet count	x	Reticulocyte count
x	Blood clotting measurements (Thromboplastin time)	x	
	(Clotting time)		
x	(Prothrombin time)	x	

b. Clinical chemistry

ELECTROLYTES		OTHER	
x	Calcium	x	Albumin
x	Chloride	x	Blood creatinine
x	Magnesium	x	Blood urea nitrogen
x	Phosphorus	x	Total Cholesterol
x	Potassium	x	Globulins
x	Sodium	x	Glucose
ENZYMES		x	Total bilirubin
x	Alkaline phosphatase (ALK)	x	Total serum protein (TP)
	Cholinesterase (ChE)	x	Triglycerides
	Creatine phosphokinase		Serum protein electrophores
	Lactic acid dehydrogenase (LDH)		
x	Serum alanine amino-transferase (also SGPT)		
x	Serum aspartate amino-transferase (also SGOT)		
x	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

6. Urinalysis*

Urine was collected overnight from animals in metabolism cages from which food and water was withdrawn. Urine was collected at approximately months 3, 6, 12, 18, and 24. The CHECKED (X) parameters were examined.

x	Appearance*	x	Glucose*
x	Volume*	x	Ketones*
x	Specific gravity*	x	Bilirubin*
x	pH	x	Blood*
x	Sediment (microscopic)*	x	Nitrite
x	Protein*	x	Urobilinogen

* Required for chronic studies

7. Sacrifice and pathology

All animals that died and those sacrificed on schedule (by decapitation under CO₂ anesthesia following a 16-24 hour fasting period) were subjected to gross pathological examination and the CHECKED (X) tissues

were collected for histological examination. The (XX) organs, in addition, were weighed.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
x	Tongue	x	Aorta	xx	Brain
x	Salivary glands	x	Heart	x	Periph. nerve
x	Esophagus	x	Bone marrow	x	Spinal cord (3 levels) ^T
x	Stomach	x	Lymph nodes	x	Pituitary
x	Duodenum	x	Spleen	x	Eyes (optic n.) ^T
x	Jejunum	x	Thymus		
x	Ileum				
x	Cecum		UROGENITAL	xx	GLANDULAR
x	Colon	xx	Kidneys ⁺		Adrenal gland
x	Rectum	x	Urinary bladder	x	Lacrimal gland ^T
xx	Liver ⁺	xx	Testes ⁺	x	Mammary gland ^T
	Gall*bladder	x	Epididymides	x	Parathyroids
x	Pancreas	x	Prostate	x	Thyroids
		x	Seminal vesicle		
	RESPIRATORY	x	Ovaries	x	OTHER
x	Trachea	x	Uterus	x	Bone
x	Lung	x	Vagina	x	Skeletal muscle
	Nose	x	Oviduct	x	Skin
	Pharynx				All gross lesions
	Larynx				and masses

⁺ Organ weight required in subchronic and chronic studies.

^T = required only when toxicity or target organ

II. RESULTS

A. OBSERVATIONS

1. Toxicity

There were no observable effects on the animals attributable to compound administration.

2. Mortality

There was no treatment-related effect on mortality in either sex of rats. The mortality rates at the end of the two-year administration period were, in males: 15%, 60%, 25%, 20%, 20%; in females: 25%, 25%, 40%, 20%, 15% at doses of 0, 200, 800, 8000, 16,000 ppm, respectively. There were no notable overall differences in the age at death of animals dying prior to sacrifice, and the increased mortality rates in 200 ppm males and 800 ppm females appeared to be spurious.

B. BODY WEIGHT AND WEIGHT GAIN

The greatest effect on body weight was seen in 8000 and 16,000 ppm females, where it was about 3-10% lower than controls throughout most of the study ($p \leq 0.05$ or 0.01). The body weights of 200 ppm and 800 ppm females were about 3-5% lower than controls from day 35 on ($p \leq 0.05$ at days

42 and 91;); some body weights at 200 ppm were greater than controls, mostly starting on day 679. The body weights of 200 and 800 ppm males were increased about 3-8% relative to controls throughout most of the 2-year study ($p > 0.05$). The body weights of the 8000 and 16,000 ppm males were about 4-6% lower than controls from day 35 to study termination; only the day 42 decrease at 16,000 ppm was significant ($p \leq 0.05$). Body weight gains paralleled the body weight changes in both sexes. In females, gains decreased roughly 3-10% ($p \leq 0.05$ or 0.01) at all doses throughout the study except at 200 ppm, where increases or decreases of 2-10% were seen ($p \leq 0.05$ or 0.01 on several days). In males, weight gain was increased about 2-6% at 200 ppm and 800 ppm ($p > 0.05$) and decreased about 3-5% at 8000 and 16,000 ppm ($p \leq 0.05$ or 0.01 on several days). With the exception of the body weight decreases in 8000 and 16,000 ppm females, which were of borderline biological significance, none of the other weight changes appeared to be biologically or toxicologically important: they were minor, not clearly dose-related, and may partly reflect a problem with compound palatability because the higher dose animals ate somewhat less food than the controls (statistical analysis was not performed). The results are shown in Table 2.

TABLE 2. Group mean body weights and body weight gains (g) in rats fed Reg. No. 242 009 for 2 years¹

Day of study	Exposure concentration (ppm)				
	0	200	800	8000	16,000
Males - Mean body weight					
42	396.6	398.0 (0.4)	395.4 (0.3)	377.1 (4.9)	375.1* (5.4)
91	482.2	491.1 (1.8)	484.1 (0.4)	457.4 (5.1)	459.5 (4.7)
203	572.8	587.0 (2.5)	582.2 (1.6)	539.0 (5.9)	550.5 (3.9)
371	651.1	677.9 (4.1)	678.3 (4.2)	610.4 (6.3)	620.9 (4.6)
539	698.2	733.0 (5.0)	743.7 (6.5)	656.7 (5.9)	672.6 (3.7)
735	711.8	756.9 (6.3)	776.2 (9.0)	666.4 (6.4)	686.4 (3.6)
Males - Mean body weight change					
371	456.5	481.0 (5.4)	481.8 (5.5)	414.2 (9.3)	428.0 (6.2)
735	517.0	558.8 (8.1)	579.2 (12.0)	469.3 (9.2)	494.2 (4.4)
Females - Mean body weight					
42	246.0	233.7* (5.0)	232.2* (5.6)	225.6** (8.3)	227.2** (7.6)
91	285.0	270.5* (5.1)	270.8* (5.0)	263.6** (7.5)	264.7** (7.1)
203	320.9	308.9 (3.7)	308.6 (3.8)	299.3** (6.7)	296.2** (7.7)
371	346.4	335.2 (3.2)	340.2 (1.8)	319.4* (7.8)	313.2** (9.6)
539	376.3	376.7 (0.1)	375.3 (0.3)	345.0 (8.3)	337.5* (10.3)
735	412.0	430.7 (4.5)	363.5 (11.8)	375.2 (8.9)	385.7 (6.4)
Females - Mean body weight change					
371	199.6	189.0 (5.3)	191.7 (4.0)	172.9* (13.4)	165.5** (17.1)

TABLE 2. Group mean body weights and body weight gains (g) in rats fed Reg. No. 242 009 for 2 years ¹					
Day of study	Exposure concentration (ppm)				
	0	200	800	8000	16,000
735	264.2	283.1 (7.2)	214.2 (18.9)	230.0 (12.9)	237.8 (10.0)

Data taken from Tables 11-20, 23, 25, 28, and 30; pp. 76-85, 88, 90, 93, and 95, MRID 43864247.

Significantly different from control: * $p \leq 0.05$; ** $p \leq 0.01$.

Bolded numbers are those whose values increased relative to controls. The numbers in parenthesis are the percent change relative to untreated controls, calculated by the reviewer.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food consumption

The food consumption of 200 and 800 ppm males was somewhat higher (about 2-6%) and of 8000 and 16,000 ppm males was slightly lower (about 3-7%) than controls. Consumption by treated females was generally about 2-9% lower than controls, with no clear dose or time-dependence. A statistical analysis of the food consumption was not performed to determine the significance of the changes, although they were relatively minor since all the values for males were within 11.1% of controls and of females were within 12.2% of controls.

2. Compound consumption

Animals were given the test compound in the diet, and its mean daily intake as a time-weighted average (mg compound/kg/day) for both sexes is given in Table 1.

3. Food efficiency

There were no notable or consistent differences between the controls and any treated group of either sex. Statistical analysis was not performed.

D. OPHTHALMOSCOPIC EXAMINATION

There were no treatment-related findings in either sex.

E. BLOOD WORK

1. Hematology

In 8000 ppm males, the MCV was slightly lowered at 24 months (3.2%, $p \leq 0.05$), and in 16,000 ppm males the MCV and MCH were lowered at 3, 6, and 12 or 24 months ($\leq 3.1\%$, $p \leq 0.05$ or 0.01). In females the MCV and MCH

were lowered and the red blood cell count was elevated at 3 and/or 6 and 12 months ($\leq 7.4\%$, $p \leq 0.05$ or 0.01) in most dose groups. The small magnitude of the changes, their transient nature and lack of dose-dependence, and similarity to historical controls indicates they were neither treatment-related nor biologically significant.

2. Clinical chemistry

Alkaline phosphatase (AP) was significantly decreased relative to controls (11.2-37.2%; $p \leq 0.05$ or 0.01) in virtually all dosed groups of both sexes throughout the two-year study. Serum alanine aminotransferase (SGPT) was depressed in both sexes at one or more time points at 800, 8000, and/or 16,000 ppm ($\leq 29.1\%$ decrease; $p \leq 0.05$ or 0.01). Gamma-glutamyl transferase (GGT) was elevated in 8000 and 16,000 ppm males in a time and dose-dependent manner throughout the study (4.8 to 46-fold, $p \leq 0.01$). The minor decreases in AP and SGPT levels were not clearly dose- or time-dependent, and were not toxicologically relevant. The increased GGT levels in males were correlated with liver histopathological changes and weight increases, and are probably treatment-related. Other clinical chemistry parameters were transiently altered during the study in one or both sexes, independently of dose (generally $< 10\%$ change, $p \leq 0.05$ or 0.01). Affected were the serum urea, albumin, triglycerides, creatinine, calcium, glucose, total protein, and globulins. The enzyme results are summarized in Table 3.

TABLE 3: Clinical chemistry changes in rats given Reg. No. 242 009 for two years						
Parameter	Day	Dose (ppm)				
		0	200	800	8000	16,000
Males						
Alkaline phosphatase (AP) (microkatal/L)	92	4.94	4.39*	3.81**	3.91**	3.76**
	179	4.47	3.96*	3.65**	3.73**	3.49**
	365	4.76	4.00**	3.67**	3.61**	3.41**
	547	4.56	3.84**	3.56**	3.39**	3.45**
	732	4.00	3.54	3.25**	3.08**	3.08**
Alanine aminotransferase (SGPT) (microkatal/L)	92	1.01	1.01	0.88*	0.79**	0.74**
	179	0.94	0.93	0.88	0.83*	0.76**
	365	1.05	1.06	0.96	0.93	0.91
	547	1.10	0.98	1.01	0.89*	0.78**
	732	0.75	0.74	0.76	0.70	0.58*
Gamma-glutamyl transferase (nanokatal/L) (GGT)	92	9	4	9	42**	48**
	179	6	7	4	37**	46**
	365	3	0	0	45**	62**
	547	10	2	9	83**	131**
	732	3	2	6	91**	146**
Females						

TABLE 3: Clinical chemistry changes in rats given Reg. No. 242 009 for two years						
Parameter	Day	Dose (ppm)				
		0	200	800	8000	16,000
Alkaline phosphatase (AP) (microkatal/L)	92	3.49	2.71**	2.53**	2.51**	2.65**
	180	3.25	2.51**	2.29**	2.16**	2.17**
	365	2.65	1.94**	1.90**	1.71**	2.35
	548	2.42	1.84**	1.74**	1.52**	1.53**
	729	2.56	1.90**	1.77**	1.65**	1.78**
Alanine aminotransferase (SGPT) (microkatal/L)	92	0.88	0.76	0.66**	0.67**	0.70**
	180	0.90	0.85	0.79	0.73	0.75
	365	0.96	0.91	0.83	0.73**	0.81
	548	0.96	0.81	0.79*	0.73**	0.68**
	729	0.88	0.70	0.74	0.82	0.75

Data taken from pages 174-183, MRID 43864247.

Significantly different from control: * $p \leq 0.05$; ** $p \leq 0.01$.

F. URINALYSIS

There were no treatment-related findings. The only statistically significant changes were an increase in specific gravity ($p \leq 0.05$ or 0.01) in all treated males at day 726, this being an artefact due to an abnormally low control value, and an increase in bacteria in the urine of 200 ppm males at day 187 ($p \leq 0.05$).

G. SACRIFICE AND PATHOLOGY

1. Organ weight

In males, the absolute weight of the liver was increased 16.3% ($p \leq 0.05$) at 16,000 ppm. The relative (to body) liver weight of males was elevated at both 8000 and 16,000 ppm by 14.7% ($p \leq 0.05$) and 21.6% ($p \leq 0.01$), respectively. The absolute weight of the adrenals was decreased in 8000 and 16,000 ppm males by about 16-19% ($p \leq 0.05$), whereas the relative adrenal weight was lowered by 20% at 800 and 16,000 ppm ($p \leq 0.05$). In females, the only statistically significant change was a slight increase in the absolute brain weight at 200 ppm (4.3%, $p \leq 0.05$), which appeared to be spurious. The liver weight increases in males were treatment-related and toxicologically relevant: they were correlated with liver histopathological changes, enzyme alterations, and an increased incidence of neoplasia, which may account for the weight increase. The decreased adrenal weight in males and increased brain weight in females were not accompanied by histopathological correlates and are not toxicologically significant. The results are shown in Table 4.

TABLE 4: Organ weights, absolute and relative-to-body, of rats given Reg. No. 242 009 for 2 years ¹					
Organ and Terminal Body Weight	Dose (ppm)				
	0	200	800	8000	16,000
Males					
Body weight (g)	684.325	729.238	746.14	637.969	655.094
Liver:					
Absolute (g)	18.872	20.485	19.855	20.229	21.946* (16.3)
Relative (%)	2.765	2.824	2.671	3.172* (14.7)	3.362** (21.6)
Adrenal glands:					
Absolute (mg)	100.125	88.625	91.000	83.875* (16.2)	81.25* (18.9)
Relative (%)	0.015	0.012	0.012* (20.0)	0.013	0.012* (20.0)
Females					
Body weight (g)	379.262	408.2	339.542	352.088	367.177
Brain:					
Absolute (g)	2.022	2.108* (4.4)	2.046	2.04	2.001
Relative (%)	0.54	0.547	0.616	0.602	0.566

Data taken from pages 969-976, MRID 43864247.

Significantly different from control: * $p \leq 0.05$; ** $p \leq 0.01$.

¹Numbers in parenthesis are the percent change relative to untreated controls, calculated by the reviewer.

2. Gross pathology

The most notable treatment-related pathological finding was an increased incidence of liver cysts in males ($p \leq 0.01$ at 16,000 ppm). The incidence was dose-related and accompanied by increased liver weights and changes in serum enzymology and liver histopathology. There was also a small increase in the incidence of liver masses in males, and of both liver cysts and masses in females that was possibly treatment-related, though was not statistically significant. Males also had an elevated incidence of kidney retraction ($p \leq 0.05$) at 16,000 ppm. In females, there were increased incidences of glandular stomach lesions at 800 ppm and ovarian cysts at 16,000 ppm ($p \leq 0.05$). The increases in glandular stomach lesions in females and kidney retraction in males had no pathological correlates and/or were not dose-dependent and appeared to be spurious. The dose-related increase in ovarian cysts in females was correlated with an equivocal increase in ovarian stromal hyperplasia ($p \leq 0.05$ only at 200 ppm), but not with ovarian neoplasia, suggesting that the ovarian cysts were not toxicologically important. The results are presented in Table 5.

TABLE 5: Incidence of macroscopic changes found in rats given Reg. No. 242 009 for 2 years ¹					
Organ - lesion	Dose (ppm)				
	0	200	800	8000	16,000
Males					
Liver - cyst	0/20	1/20	0/20	3/20	6/20**
- mass	1/20	1/20	1/20	4/20	2/20
Kidneys - retraction	1/20	1/20	2/20	0/20	7/20*
Females					
Liver - cyst	2/20	7/20	5/20	5/20	4/20
- mass	0/20	0/20	1/20	2/20	3/20
Glandular stomach - lesión	0/20	3/20	5/20*	1/20	2/20
Ovaries - cyst	2/20	3/20	4/20	7/20	8/20*

Data were taken from pages 977-986, MRID 43864247.

¹The incidences were statistically analyzed by the reviewer using the Fisher exact test. Values significantly different from controls are designated: *p ≤ 0.05; **p ≤ 0.01.

3. Microscopic pathology

- a) Non-neoplastic - There was a dose-related increase in males in the incidence of liver eosinophilic foci, mixed cell foci, and cellular hypertrophy that was statistically significant at 8000 and/or 16,000 ppm ($p \leq 0.05$ or 0.01). Females also had increased hepatocellular hypertrophy at 16,000 ppm ($p \leq 0.01$), and had several incidences of eosinophilic and/or mixed cell foci. The liver alterations were correlated with liver weight increases (males), macroscopic lesions (cysts, masses), serum enzyme changes, and an increased incidence of hepatocellular neoplasia in both sexes (see section II.C.3.b.). Other non-neoplastic findings include, in females: ovarian stromal hyperplasia (200 ppm; $p \leq 0.05$), mandibular lymph node erythrophagocytosis (16,000 ppm; $p \leq 0.05$), and kidney tubular casts (16,000 ppm, $p \leq 0.01$) and, in males: adrenal cortex angiectasis (800, 8000 ppm, $p \leq 0.01$). The toxicological relevance and etiology of these changes was equivocal because they were not clearly dose-related or of marginal significance, and lacked relevant pathological correlates. The histopathology results are shown in Table 6.

TABLE 6: Incidence of microscopic findings in rats given Reg. No. 242 009 for 2 years ¹					
Organ: lesion	Dose (ppm)				
	0	200	800	8000	16,000
Males					
Liver:					
eosinophilic foci	0/20	1/20	0/20	6/20**	8/20**
mixed cell foci	0/20	0/20	2/20	4/20	5/20*
cellular hypertrophy	0/20	0/20	3/20	4/20	7/20**
Adrenal cortex:					
angiectasis	2/20	3/20	10/20**	14/20**	4/20
Females					
Liver:					
eosinophilic foci	1/20	0/20	0/20	0/20	1/20
mixed cell foci	0/20	0/20	0/20	0/20	2/20
cellular hypertrophy	1/20	1/20	0/20	1/20	8/20**
Ovaries:					
stromal hyperplasia	3/20	10/20*	4/20	8/20	8/20
Kidneys: tubular casts	2/20	1/20	2/20	6/20	10/20* *
Mandibular lymph nodes:					
erythrophagocytosis	0/19	0/5	2/8	0/4	6/20*

Data were taken from pages 1002-1020, MRID 43864247.

¹The incidences were statistically analyzed by the reviewer using the Fisher exact test. Values significantly different from controls are designated: *p ≤ 0.05; **p ≤ 0.01.

- b) Neoplastic - The most significant treatment-related neoplastic finding was a dose-related increased incidence of hepatocellular carcinoma in both sexes at 8000 and 16,000 ppm, with statistical significance being achieved only in 16,000 ppm males (p ≤ 0.01). Historic controls provided by the performing laboratory indicate that the carcinoma incidence of the present female control group (5%) was at the very upper end of the range obtained, lending support to the significance of the carcinoma in 8000 and 16,000 ppm females. The liver carcinoma was correlated with increased liver weight and microscopic lesions. Liver cholangioma and/or cholangiocarcinoma was also seen in a few animals (0 to 2 per dose group); it is unclear whether these were treatment-induced. These results are presented in Table 7. Spontaneous neoplasms (incidence comparable to controls) were found in both sexes in many other organs. The overall number of tumor-bearing animals and the number of primary neoplasms were comparable in controls and

treated groups; however, the number of malignant tumors was somewhat elevated in 8000 ppm females and in 16,000 ppm males and females (not statistically significantly; see pp. 1086-1087, MRID 43864247).

TABLE 7: Incidence of liver neoplasms in rats given Reg. No. 242 009 for 2 years ¹					
Liver neoplasm	Dose (ppm)				
	0	200	800	8000	16,000
Males					
Adenoma	0/20	0/20	0/20	0/20	0/20
Carcinoma	0/20	1/20	1/20	3/20	8/20 ⁺⁺
Cholangioma	0/20	0/20	0/20	0/20	1/20
Cholangiocarcinoma	0/20	2/20	0/20	0/20	0/20
Females					
Adenoma	0/20	0/20	0/20	0/20	0/20
Carcinoma	1/20	0/20	2/20	6/20	6/20
Cholangioma	0/20	0/20	0/20	1/20	0/20

Data were taken from pages 963 and 1052-1053, MRID 43864247.

¹Statistical significance was based on Chi-Squared analysis. The p-value ⁺⁺ represents $p \leq 0.01$.

III. DISCUSSION

A. DISCUSSION

In a chronic toxicity study (MRID 43864247) Reg. No. 242 009 was administered to 20 Wistar rats/sex/dose in the feed at doses of 0, 200, 800, 8000, and 16,000 ppm (mean compound intake in males was 0, 9, 36, 370, and 746 mg/kg/day and for females was 0, 12, 48, 503, 985 mg/kg/day, respectively. Food and water were provided *ad libitum*. The rats were examined once or twice daily for signs of toxicity and mortality. They were weighed before the start of the administration period, once a week during the first 13 weeks, and every four weeks thereafter. Blood was collected at approximately months 3, 6, 12, 18, and 24 from all surviving animals to measure hematology and clinical chemistry parameters.

There was no treatment-related effect on animal mortality in either sex of rats. The greatest effect on body weight occurred in 8000 and 16,000 ppm females, where it was 3-10% lower than in controls throughout the study ($p \leq 0.05$ or 0.01) and was of borderline toxicological significance. The minor body weight decreases in the 200 and 800 ppm females and 8000 and 16,000 ppm males (3-6%, generally $p > 0.05$) were not biologically relevant. The small body weight increases in one or both sexes at 200 and 800 ppm (about 3-8%, $p > 0.05$) appeared to be incidental to treatment. Body weight gains paralleled body weight

changes in both sexes. The lowered weight gains seen in both sexes at 8000 and 16,000 ppm may have been partly due to a palatability problem with the test compound because these animals appeared to have a somewhat lowered food intake (statistical analysis was not performed) and the food efficiency was not affected. There were small alterations ($\leq 7.4\%$ change, $p \leq 0.05$ or 0.01) in several hematology parameters in one or both sexes (MCV, MCH, red blood cells) that were transient, dose-independent, and not toxicologically relevant.

The most toxicologically significant clinical chemistry finding was a time and dose-dependent increase in gamma-glutamyl transferase (GGT) in 8000 and 16,000 ppm males throughout the study (4.8 to 46-fold, $p \leq 0.01$). This was correlated with increased liver weight and numerous pathological findings. The levels of serum alkaline phosphatase (AP) and serum alanine aminotransferase (SGPT) were decreased relative to controls ($\leq 38\%$; $p \leq 0.05$ or 0.01) in most dose groups of both sexes throughout the two-year study. The decreased AP and SGPT levels were not clearly dose or time-dependent, and of dubious toxicological significance. An independent study of the origins and pathological significance of the AP and SGPT decreases by D.W. Moss (pp. 1703-1726 of MRID 43864247) concluded that they were not toxicologically relevant and were "probably due to a slight alteration in food resorption in treated animals." Other clinical chemistry parameters altered during the study in one or both sexes (serum urea, albumin, triglycerides, creatinine, calcium, glucose, total protein, and globulins) were neither treatment-related nor toxicologically relevant.

In addition to a dose-related increase in absolute and/or relative liver weight (16-22%, $p \leq 0.05$ or 0.01), 8000 and 16,000 ppm males had decreased absolute and/or relative adrenal weight (16-20%, $p \leq 0.05$). There was also an increase in the absolute brain weight of 200 ppm females (4.3%, $p \leq 0.05$). The adrenal and brain weight changes, unlike that of the liver, had no pathological correlates and were not toxicologically significant. The brain weight change appeared to be spurious; the etiology of the adrenal weight change is unknown.

The macroscopic and microscopic pathological results implicate the liver as a target organ in both sexes, consistent with the clinical enzymology and organ weight alterations. There was a dose-related increase in the incidence of liver cysts in 16,000 ppm males ($p \leq 0.01$) and minor increases in liver cysts and masses in both sexes at 8000 and 16,000 ppm ($p > 0.05$). These were correlated in males with an increased incidence of liver eosinophilic foci, mixed cell foci, and cellular hypertrophy at 8000 and/or 16,000 ppm and in females with cellular hypertrophy at 16,000 ppm ($p \leq 0.05$ or 0.01).

The ovarian pathologies that were found (hyperplasia, $p \leq 0.05$ at 200 ppm; cysts, $p \leq 0.05$ at 16,000 ppm) were probably not toxicologically relevant because they were not correlated with neoplasia and were statistically significant at 40-fold different doses. Other pathological findings in males (kidney retraction, adrenal cortex angiectasis) and females (glandular stomach lesions, mandibular lymph node erythrophagocytosis, kidney tubular casts) were of equivocal etiology and toxicological significance because they lacked other pathological correlates and/or were not clearly dose-related.

The LOEL for both male and female rats is 8000 ppm (about 370 mg/kg/day in males and 503 mg/kg/day in females). For males, this is based on the increase in SGGT levels, liver weight and histopathological changes. In females, this is based on the roughly 10% lowered body weights and weight gains throughout much of the study. The NOEL for both sexes is 800 ppm, corresponding to about 36 mg/kg/day in males and 48 mg/kg/day in females.

Significant treatment-related neoplastic findings were confined to the liver in both sexes of rats. There was a dose-related increase in the incidence of hepatocellular carcinoma in both males and females at 8000 and 16,000 ppm. It was statistically significant only in 16,000 ppm males ($p \leq 0.01$), but was most likely also treatment-induced in 8000 and 16,000 ppm females. This assertion is supported by historic controls provided by the performing laboratory (BASF Department of Toxicology) indicating that the incidence of carcinoma in the present study female control group (5%) was seven-fold higher than the average incidence in 29 studies (0.7%). As it was not possible to obtain an incidence $< 5\%$ in the present study (other than zero) due to sample size, a statistically significant increase in liver carcinoma might have been seen with a larger sample size or in a repeat experiment. Additionally, in a 2-year oncogenicity study performed using the same doses and strain of rats (MRID 43864249), an increase in liver carcinoma was seen in females at 8000 ppm. The relationship to treatment of the sporadically seen liver cholangioma and/or cholangiocarcinoma is unknown. Many other organs had various spontaneous neoplasms with an incidence comparable to that of controls. Although the overall number of primary neoplasms and tumor-bearing animals was comparable in control and treated groups, the total number of malignant tumors was increased somewhat in 8000 ppm females and in both sexes at 16,000 ppm (not statistically analyzed), possibly due to the liver neoplasms. Although it is possible that the animals could have tolerated a higher dose of test compound (intake of 746 mg/kg/day for males and 985 mg/kg/day for females at 16,000 ppm), the intake for females approximated the highest dose recommended for testing according to EPA guidelines (1000 mg/kg/day).

In a separate study (MRID 43864246; summarized in the Appendix), the effect of Reg. No. 242 009 on hepatic cell proliferation was investigated. In this experiment, 5 male rats/dose were given 0, 200, or 16,000 ppm (0, 15, 1140 mg/kg/day, respectively) in the feed for 3 weeks, during the last week also being given bromodeoxyuridine from subcutaneously implanted osmotic minipumps. No treatment-related effects were seen (body weight, food consumption, clinical observations, liver weight, gross or microscopic lesions) except for a statistically significant increase (2 to 3-fold) in cell proliferation in the hepatic lobules of the 16,000 ppm group. This result confirms the present study (MRID 43864247) in identifying the liver as the target organ for toxicity, and suggests that hepatocellular carcinoma may have been developing in the 16,000 ppm males (and possibly females) after as little as three weeks of treatment.

B. STUDY DEFICIENCIES

There were no major deficiencies that would alter the interpretation or classification of this study as being acceptable. Minor deficiencies include failure to statistically analyze the food consumption and efficiency, test substance intake, differential blood count, the incidence of gross lesions, and failure to report most of statistical analysis of the histopathological results. The selection of doses for the study appeared to be suboptimal, with a 10-fold difference in the two middle doses (800 and 8000 ppm) and only a 2-fold difference between the top two doses (8000 and 16,000 ppm). There were many results that appeared to be 2-tiered, i.e. that of the lower two doses was about the same, and that of the top two doses was about the same (e.g. body weights/gains, clinical chemistry, microscopic findings), and the reviewer would have felt more comfortable asserting the presence/absence of a dose-response if the spread of doses was more even. The ability to establish a significant neoplastic response in female livers would have been improved if more animals were included in the study and if a higher maximum treatment dose was used, although in the context of the present study requirements (chronic toxicity), and the fact that the dose to the females approached 1000 mg/kg, this was not really a study deficiency.